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     (FILE 'HOME' ENTERED AT 14:21:49 ON 15 JUL 2003)

     INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
     CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
     DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:22:00 ON
     15 JUL 2003
               SEA ENDOGLUCANASE
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                1   FILE ADISCTI
              531   FILE AGRICOLA
               15   FILE ANABSTR
               25   FILE AQUASCI
              341   FILE BIOBUSINESS
               13   FILE BIOCOMMERCE
             1818   FILE BIOSIS
              862   FILE BIOTECHABS
              862   FILE BIOTECHDS
              854   FILE BIOTECHNO
              676   FILE CABA
               15   FILE CANCERLIT
             2876   FILE CAPLUS
              442   FILE CEABA-VTB
                7   FILE CIN
               29   FILE CONFSCI
                2   FILE CROPB
               16   FILE CROPU
             1780   FILE DGENE
                1   FILE DRUGU
                7   FILE EMBAL
              840   FILE EMBASE
              629   FILE ESBIOBASE
               32   FILE FEDRIP
                1   FILE FOREGE
               80   FILE FROSTI
              520   FILE FSTA
             1048   FILE GENBANK
                2   FILE HEALSAFE
              192   FILE IFIPAT
              115   FILE JICST-EPLUS
                4   FILE KOSMET
              985   FILE LIFESCI
              888   FILE MEDLINE
               40   FILE NTIS
                7   FILE OCEAN
              844   FILE PASCAL
                1   FILE PHIN
               15   FILE PROMT
                1   FILE RDISCLOSURE
             1683   FILE SCISEARCH
              270   FILE TOXCENTER
              703   FILE USPATFULL
               22   FILE USPAT2
               19   FILE VETU
              170   FILE WPIDS
              170   FILE WPINDEX
L1               QUE ENDOGLUCANASE
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     FILE 'CAPLUS, BIOSIS, SCISEARCH, LIFESCI, MEDLINE, BIOTECHDS, BIOTECHNO,
     PASCAL, EMBASE' ENTERED AT 14:23:16 ON 15 JUL 2003
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L2	1152	S L1 AND (MUTANT OR VARIANT)
L3	8	S L2 AND (Y245G OR Y42R OR W82R)
L4	2	DUP REM L3 (6 DUPLICATES REMOVED)
L5	50989	S L1 AND (ENHANC?(W)ACTIVITY) OR (INCREAS?(W)ACTIVITY)
L6	50964	S L2 AND (ENHANC?(W)ACTIVITY) OR (INCREAS?(W)ACTIVITY)
L7	2975	S L6 AND (MUTANT OR VARIANT)
L8	0	S L7 AND (HYDROPHOBIC SURFACE BINDING)
L9	9	S L7 AND CELLOBIOSE
L10	3	DUP REM L9 (6 DUPLICATES REMOVED)
L11	0	S L7 AND ACIDOTHERMUS
L12	98	S L1 AND ACIDOTHERMUS
L13	23	S L12 AND (MUTANT OR VARIANT)
L14	10	DUP REM L13 (13 DUPLICATES REMOVED)

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L14 ANSWER 1 OF 10 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-11426 BIOTECHDS
TITLE: Novel thermal tolerant mannanase A polypeptide derived from **Acidothermus** cellulolyticus, useful for reducing hemicellulose in a starting material, for processing of food, and as bulking agents in food stuffs; vector-mediated gene transfer and expression in host cell for recombinant enzyme production
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 20030012110 13 Feb 2003
APPLICATION INFO: WO 2001-US23819 28 Jul 2001
PRIORITY INFO: WO 2001-23819 28 Jul 2001; WO 2001-23819 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-248182 [24]
AB DERWENT ABSTRACT:

NOVELTY - An isolated thermal tolerant mannanase A polypeptide (I) derived from **Acidothermus** cellulolyticus, comprising a sequence (S1) of 762 amino acids, and the fragments of (I) such as catalytic domain glycoside hydrolase (GH) 5, carbohydrate binding domain (CBD) type III, and CBD type II, having 411, 608, and 762 amino acids, respectively, is new. All sequences are given in the specification.

DETAILED DESCRIPTION - An isolated thermal tolerant mannanase A polypeptide (I) derived from **Acidothermus** cellulolyticus, comprising a sequence of 762 amino acids. The fragments of (I) such as catalytic domain, carbohydrate binding domain type III, and carbohydrate binding domain type II, have 411, 608, and 762 amino acids, respectively; or a sequence having at least 70% identity with the above sequences. All sequences are defined in the specification. INDEPENDENT CLAIMS are also included for: (1) a composition (II) comprising (I); (2) an industrial mixture suitable for degrading hemicellulose, comprising (I); (3) an isolated polynucleotide molecule (III) comprising a nucleic acid sequence having 90% sequence identity to a sequence encoding S1, or encoding a heterologous protein in frame with S1; (4) a mannanase substrate complex comprising (I) bound to hemicellulose; (5) a vector comprising (III); (6) a host cell genetically engineered to express (III); (7) a composition comprising (I) and a carrier; (8) an isolated antibody (IV) that specifically binds to (I); (9) production of (I); (10) a set of amplification primers (V) for amplification of a polynucleotide molecule encoding mannanase A, comprising two or more sequences having 9 or more contiguous nucleic acids derived from (III); and(11) a probe (VI) for hybridizing to a polynucleotide encoding mannanase A comprising a sequence of 9 or more contiguous nucleic acid derived from (III).

WIDER DISCLOSURE - Also disclosed are: (1) recombinant forms of (I); (2) **variants**, derivatives and fusion proteins of (I); and (3) reagents, compositions, and methods that are useful for analysis of ManA activity.

BIOTECHNOLOGY - Preparation: (I) is produced by incubating the above mentioned host cell (claimed). Preferred Mixture: The industrial mixture further comprises a detergent.

USE - (V) is useful for the detection of a mannanase A polynucleotide, by amplifying a nucleic acid sequence with (V), and correlating the amplified nucleic acid sequence with detected polynucleotide encoding mannanase A. (I) is useful for assessing the carbohydrate degradation activity of mannanase A, by analyzing a carbohydrate degradation in the presence of mannanase A and a carbohydrate degradation in the absence of mannanase A on a substrate, and comparing the carbohydrate degradation in the presence of mannanase A with the carbohydrate degradation in the absence of mannanase A. The method is also carried out in the presence of a desired agent. (I) is also useful for reducing hemicellulose in a starting material (claimed),

to simpler carbohydrate units, ultimately to sugars which are useful in the food, feed, paper pulp, and biofuels industries. (I) is useful for the processing of food and in food stuffs as bulking agents. Fragments of (I) are useful to generate specific anti-ManA antibodies. (I) is also useful to raise polyclonal and monoclonal antibodies that are useful in purifying ManA, or detecting ManA polypeptide expression, and as well as reagent tool for characterizing the molecular actions of ManA polypeptides. (II) is useful for removal of hemicellulose containing stains within fabrics and in pulp and paper industry to address conditions associated with hemicellulose contamination of the cellulose fraction. (II) is also useful to produce oligosaccharide bulking agents and stabilizers from hemicellulose for use in the food and feed industry.

EXAMPLE - Molecular cloning of mannanase A (ManA) was as follows. Genomic DNA was isolated from **Acidothermus** cellulolyticus and purified. The purified genomic DNA was then digested and separated on agarose gels. DNA fragments in the range of 9-20 kilobase pairs were isolated from the gels. This purified genomic DNA was ligated into the BamH1 acceptor site of purified EMBL3 lambda phage arms. Phage DNA was packaged and plated with Escherichia coli LE392 in top agar which contained the soluble cellulose analog, and carboxymethylcellulose. The plates were then incubated overnight to allow transfection, bacterial growth, and plaque formation. Plates were stained followed by destaining. lambda plaques harboring **endoglucanase** clones showed up as unstained plaques. lambda clones which screened positive were purified. Individual phage isolates were named SL-1, SL-2, SL-3, and SL-4. Subsequent subcloning efforts employed the SL-3 clone which contained 14.2 kb fragment of A. cellulolyticus genomic DNA. Template DNA was constructed using a 9 kb BamH1 fragment obtained from 14.2 kb lambda clone SL3 prepared from A. cellulolyticus genomic DNA. The 9-kb BamHI fragment from SL3 was subcloned into pDR540 to generate a plasmid NREL501. NREL501 was first sequenced, then subcloned into pUC19, and transformed into E. coli XL1-blue for the production of template DNA for sequencing. Each subclone was sequenced, and the sequencing data from primer walking and subclones were assembled together to verify that all SL3 regions had been sequenced. An open reading frame (ORF) was found in the 9-kb BamHI fragment, C-terminal of E1, and then termed as ManA. An ORF of 2289 bp defined in the specification and its deduced amino acid sequences having 762 amino acids were obtained. The amino acid sequence predicted was determined to have significant homology to known mannanases.(46 pages)

L14 ANSWER 2 OF 10 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-11180 BIOTECHDS
TITLE: Novel thermal tolerant GuxA polypeptide derived from **Acidothermus** cellulolyticus, useful for reducing cellulose in a starting material, and for the conversion of biomass to biofuels and biofuel additives; vector-mediated recombinant protein gene transfer and expression in host cell for use in carbohydrate degradation, cellulose degradation, fuel, surfactant, paper mill and monoclonal antibody preparation
AUTHOR: DING S; ADNEY W S; VINZANT T B; HIMMEL M E; DECKER S R
PATENT ASSIGNEE: MIDWEST RES INST
PATENT INFO: WO 20030012109 13 Feb 2003
APPLICATION INFO: WO 2001-US23817 28 Jul 2001
PRIORITY INFO: WO 2001-23817 28 Jul 2001; WO 2001-23817 28 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-239526 [23]
AB DERWENT ABSTRACT:

NOVELTY - An isolated thermal tolerant GuxA polypeptide (I) derived from **Acidothermus** cellulolyticus, having a sequence (S1) of 1228 amino acids (aa). Fragments of (I) e.g., first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (CBD)

type III, and CBD type II, having 423, 231, 150, and 101 aas, respectively, is new. All sequences are fully disclosed in the specification.

DETAILED DESCRIPTION - An isolated thermal tolerant GuxA polypeptide (I) derived from **Acidothermus** cellulolyticus, having a sequence (S1) of 1228 amino acids (aa). Fragments of (I) e.g., first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (CBD) type III, and CBD type II, having 423, 231, 150, and 101 aas, respectively. (I) has a sequence (S1) of 1228 aas. The fragments of (I) such as first catalytic domain glycoside hydrolase (CD-GH) 6, second CD-GH 12, carbohydrate binding domain (CBD) type III, and CBD type II, having 423, 231, 150, and 101 aas, respectively; or a sequence having at least 70% identity with the above sequences. INDEPENDENT CLAIMS are also included for the following; (1) composition (II) comprising (I); (2) an industrial mixture suitable for degrading cellulose, comprising (I); (3) fusion protein (III) comprising (I) and a heterologous peptide; (4) cellulase-substrate complex comprising (I) bound to cellulose; (5) vector comprising the polynucleotide that encodes (I); (6) host cell genetically engineered to express (I); (7) composition comprising (I) and a carrier; (8) isolated antibody (IV) that specifically binds to (I); (9) production of (I); (10) set of amplification primers (V) for amplification of a polynucleotide molecule encoding GuxA, comprising two or more sequences having 9 or more contiguous nucleic acids derived from the polynucleotide molecule; and (11) probe (VI) for hybridizing to a polynucleotide encoding GuxA comprising a sequence of 9 or more contiguous nucleic acid derived from the polynucleotide molecule.

WIDER DISCLOSURE - Also disclosed as new are the following: (1) recombinant forms of (I); (2) **variants**, and derivatives of (I); (3) reagents, compositions, and methods that are useful for analysis of GuxA activity; and (4) a polynucleotide encoding (I).

BIOTECHNOLOGY - Preparation: (I) is produced by incubating the above mentioned host cell (claimed). Preferred Mixture: The industrial mixture further comprises a detergent. Preferred Fusion Protein: The heterologous peptide is a leucine zipper.

USE - The set of primers (V) are useful for the detection of a polynucleotide encoding GuxA, by amplifying a nucleic acid sequence with (V), and correlating the amplified nucleic acid sequence with detected polynucleotide encoding GuxA. (I) is useful for assessing the carbohydrate degradation activity of Gux A, by analyzing a carbohydrate degradation in the presence of GuxA and a carbohydrate degradation in the absence of GuxA on a substrate, and comparing the carbohydrate degradation in the presence of GuxA with the carbohydrate degradation in the absence of GuxA. The method is also carried out in the presence of desired agent. (I) is useful for reducing cellulose in a starting material such as agricultural biomass (all claimed), to sugars which is useful in biofuel production. (I) is useful in the conversion of biomass to biofuels and biofuel additives, in detergents pulp and paper processing, food and feed processing, and in textile process. Fragments of (I) are useful to generate specific anti-GuxA antibodies. (I) is also useful to raise polyclonal and monoclonal antibodies that are useful in purifying GuxA, or detecting GuxA polypeptide expression, and as well as reagent tool for characterizing the molecular actions of GuxA polypeptides.

EXAMPLE - Molecular cloning of GuxA was as follows: Genomic DNA was isolated from **Acidothermus** cellulolyticus and purified. The purified genomic DNA was then digested and separated on agarose gels. DNA fragments in the range of 9-20 kilobase pairs were isolated from the gels. This purified genomic DNA was ligated into the BamH1 acceptor site of purified EMBL3 lambda phage arms. Phage DNA was packaged and plated with Escherichia coli LE392 in top agar which contained the soluble cellulose analog, and carboxymethylcellulose. The plates were then incubated overnight to allow transfection, bacterial growth, and plaque formation. Plates were stained followed by destaining. lambda plaques harboring **endoglucanase** clones showed up as unstained plaques.

lambda clones which screened positive were purified. Individual phage isolates were named SL-1, SL-2, SL-3, and SL-4. Subsequent subcloning efforts employed the SL-3 clone which contained 14.2 kb fragment of A.cellulolyticus genomic DNA. Template DNA was constructed using a 9 kb BamH1 fragment obtained from 14.2 kb lambda clone SL3 prepared from A.cellulolyticus genomic DNA. The 9-kb BamHI fragment from SL3 was subcloned into pDR540 to generate a plasmid NREL501. NREL501 was first sequenced, then subcloned into pUC19, and transformed into E.coli XL1-blue for the production of template DNA for sequencing. Each subclone was sequenced, and the sequencing data from primer walking and subclones were assembled together to verify that all SL3 regions had been sequenced. An open reading frames (ORF) was found in the 9-kb BamHI fragment, C-terminal of E1, and then termed as GuxA. An ORF of 3687 bp defined in the specification and its deduced aa sequences having 1228 aas were obtained. The aa sequence predicted was determined to have significant homology to known cellulases. (47 pages)

L14 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:323635 CAPLUS
DOCUMENT NUMBER: 137:43353
TITLE: Effect of single active-site cleft mutation on product specificity in a thermostable bacterial cellulase
AUTHOR(S): Rignall, Tauna R.; Baker, John O.; McCarter, Suzanne L.; Adney, William S.; Vinzant, Todd B.; Decker, Stephen R.; Himmel, Michael E.
CORPORATE SOURCE: Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA
SOURCE: Applied Biochemistry and Biotechnology (2002), 98-100(Biotechnology for Fuels and Chemicals), 383-394
CODEN: ABIBDL; ISSN: 0273-2289
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutation of a single active-site cleft tyrosyl residue to a glycyl residue significantly changes the mixt. of products released from phosphoric acids-wollen cellulose (PSC) by EIcd, the catalytic domain of the **endoglucanase**-I from **Acidothermus** cellulolyticus. The percentage of glucose in the product stream is almost 40% greater for the Y245G **mutant** (and for an addnl. double **mutant**, Y245G/Q204A) than for the wild type enzyme. Comparisons of results for digestion PSC and of pretreated yellow poplar suggest that the obsd. shifts in product specificity are connected to the hydrolysis of a more easily digestible fraction of both substrates. A model is presented that relates the changes in product specificity to a mutation-driven shift in indexing of the polymeric substrate along the extended binding-site cleft.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 2002:323625 CAPLUS
DOCUMENT NUMBER: 137:43352
TITLE: Exploration of cellulose surface-binding properties of **Acidothermus** cellulolyticus Cel5A by site-specific mutagenesis
AUTHOR(S): McCarter, Suzanne L.; Adney, William S.; Vinzant, Todd B.; Jennings, Edward; Eddy, Fannie Posey; Decker, Stephen R.; Baker, John O.; Sakon, Joshua; Himmel, Michael E.
CORPORATE SOURCE: Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA
SOURCE: Applied Biochemistry and Biotechnology (2002), 98-100(Biotechnology for Fuels and Chemicals), 273-287

CODEN: ABIBDL; ISSN: 0273-2289
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Understanding the interactions between cellulases and cellulosic substrates is crit. to the development of an efficient artificial cellulase system for conversion of biomass to sugars. We directed specific mutations to the interactive surface of the **Acidothermus** cellulolyticus EI **endoglucanase** catalytic domain. The cellulose-binding domain is not translated in these **mutants**. Amino acid mutations were designed either to change the surface charge of the protein or to modify the potential for hydrogen bonding with cellulose. The relationship between cellulase-to-cellulose (Avicel PH101) binding and hydrolysis activity was detd. for various groupings of mutations. While a significant increase in hydrolysis activity was not obsd., certain clusters of residues did significantly alter substrate binding and some interesting correlations emerged. In the future, these observations may be used to aid the design of **endoglucanases** with improved performance on pretreated biomass.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:229028 CAPLUS
DOCUMENT NUMBER: 134:248007
TITLE: Downstream box **variants** for use in increasing the efficiency of translation of foreign genes in plastids
INVENTOR(S): Chaudhuri, Sumita
PATENT ASSIGNEE(S): Calgene LLC, USA
SOURCE: PCT Int. Appl., 52 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001021782	A2	20010329	WO 2000-US26052	20000922
WO 2001021782	A3	20020103		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

EP 1214434	A2	20020619	EP 2000-963724	20000922

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

PRIORITY APPLN. INFO.: US 1999-156071P P 19990924
WO 2000-US26052 W 20000922

AB Elements that can be used to increase the efficiency of translation of foreign genes on plastid ribosomes are described. Specifically, **variants** of the downstream box (DB) that lies 3' of the Shine-Dalgrano sequence and that is involved in interaction with the 16S rRNA in the ribosome are described. A series of **variants** of known downstream boxes were generated and tested for their effects on the level of expression of a bacterial gene (the .beta.-1,4-**endoglucanase** gene of **Acidothermus** E1) from a bacteriophage T7 promoter in tobacco plastids. A clear effect of the DB on the efficiency of translation was obsd.

L14 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:824394 CAPLUS
DOCUMENT NUMBER: 134:2062
TITLE: **Acidothermus** cellulolyticus E1 **endoglucanase variants** Y245G, Y82R and W42R with increased catalytic activity
INVENTOR(S): Himmel, Michael E.; Adney, William S.; Baker, John O.; Vinzant, Todd B.; Thomas, Steven R.; Sakon, Joshua; Decker, Stephen R.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000070031	A1	20001123	WO 2000-US13971	20000519
WO 2000070031	C2	20020704		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1179051	A1	20020213	EP 2000-937647	20000519
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 2003054535	A1	20030320	US 2001-997504	20011119

PRIORITY APPLN. INFO.: US 1999-134925P P 19990519
WO 2000-US13971 W 20000519

AB The invention provides a method for making a glycosyl hydrolase characterized by an increase in catalytic activity on an insol. substrate. An active site assocd. glycosyl-stabilizing amino acid of the hydrolase is thus replaced with an amino acid, the replacing amino acid not strongly binding a disaccharide product in the active site. The method for making a glycosyl hydrolase characterized by an increase in catalytic activity on a sol. substrate comprises replacing a hydrophobic substrate binding amino acid of the hydrolase with a pos. charged amino acid. The invention specifically provides **Acidothermus** cellulolyticus E1 **endoglucanase variants**, comprising Y42R, W82R, or Y245G, and the DNA sequences encoding the enzymes. Ki values for inhibition of hydrolysis of 4-.beta.-D-cellobioside by native and Y245G **mutant** E1 indicate that the **mutant** catalytic domain binds cellobiose 15-fold less tightly than does the native enzyme, i.e., an increase in Ki from 2 to 30 mM cellobiose and a decrease in apparent binding energy of 1.7 kcal/mol.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 7 OF 10 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1999-00710 BIOTECHDS
TITLE: Engineering cellulase systems: enzyme modifications for improved function;
recombinant thermostable **endoglucanase** production, purification and characterization by Escherichia coli (conference abstract)
AUTHOR: Himmel M E; Adney W S; Baker J O; Decker S R; Vinzant T B; Nieves R A; Godbole S; Thomas S R

CORPORATE SOURCE: Nat.Renewable-Energy-Lab.Colorado
LOCATION: National Renewable Energy Laboratory, Golden, CO, USA.
SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1998) 98 Meet., 28
CODEN: 0005P
ISSN: 0067-2777
98th General Meeting of the American Society for Microbiology, Atlanta, GA, USA, 17-21 May, 1998.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In general, cellulase (EC-3.2.1.4) systems of interest to industry would be more cost effective if they displayed higher specific activities. **Acidothermus** cellulolyticus EI is a thermotolerant **endoglucanase**, which was isolated from a hot spring bacterium that demonstrated very high synergism with fungal cellobiohydrolase (EC-3.2.1.91). A recent 1.8 Angstrom crystallographic structure was the basis of the current strategy to improve the EI catalytic domain (cd) by polymerase chain reaction mutation. Modifications were targeted towards areas of the enzyme that could improve its saccharification of biomass. Polymerase chain reaction was used to generate 18 **mutant** EI coding sequences and following verification of the mutation sites by polymerase chain reaction DNA sequencing, microtiter plate assays were employed to determine that 13 of these **mutants** yielded active enzymes. Transformed Escherichia coli, which expressed this enzyme, was then grown and each active **mutant** enzyme was purified to homogeneity using a novel 3-step column chromatographic method. (0 ref)

L14 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 1996:262842 CAPLUS
DOCUMENT NUMBER: 124:309585
TITLE: Gene coding for the E1 **endoglucanase** from **Acidothermus** cellulolyticus
INVENTOR(S): Thomas, Steven R.; Laymon, Robert A.; Himmel, Michael E.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: PCT Int. Appl., 33 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9602551	A1	19960201	WO 1995-US8868	19950714
W: AU, BR, CA, CN, DE, ES, GB, JP, KP, KR, NZ, SE				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5536655	A	19960716	US 1994-276213	19940715
AU 9530985	A1	19960216	AU 1995-30985	19950714
AU 682125	B2	19970918		
EP 771321	A1	19970507	EP 1995-926694	19950714
R: DE, ES, FR, GB				

PRIORITY APPLN. INFO.:

US 1994-276213	19940715
US 1989-412434	19890926
US 1992-826089	19920127
US 1993-125115	19930921
WO 1995-US8868	19950714

AB The gene encoding **Acidothermus** cellulolyticus E1 **endoglucanase** was cloned and expressed in heterologous microorganisms by std. recombinant DNA techniques. The 3004-bp fragment of DNA contains a coding portion of 1686 bp corresponding to a deduced protein sequence of 562 amino acids and contg. a 41-residue signal moiety. The gene architecture is similar to that of cellulase genes isolated from other actinomycete bacteria. New modified E1 **endoglucanase** enzymes are produced along with **variants** of the gene and enzyme.

The E1 **endoglucanase** is useful for hydrolyzing cellulose to sugars for simultaneous or later fermn. into alc. Recombinant prodn. (e.g., in Escherichia coli or Saccharomyces lividans) result in much improved rate of of enzyme prodn., thereby lowering the cost of cellulase and the prodn. of alc. using cellulosic materials as substrate.

L14 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1996:467404 CAPLUS
DOCUMENT NUMBER: 125:107081
TITLE: Gene encoding the E1 **endoglucanase** from **Acidothermus** cellulolyticus
INVENTOR(S): Thomas, Steven R.; Laymon, Robert A.; Himmel, Michael E.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: U.S., 21 pp., Cont.-in-part of U.S. 5, 366, 884.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 8
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5536655	A	19960716	US 1994-276213	19940715
US 5110735	A	19920505	US 1989-412434	19890926
EP 885955	A2	19981223	EP 1998-108104	19900827
EP 885955	A3	19990407		
R: DE, FR, GB				
US 5275944	A	19940104	US 1992-826089	19920127
US 5366884	A	19941122	US 1993-125115	19930921
WO 9602551	A1	19960201	WO 1995-US8868	19950714
W: AU, BR, CA, CN, DE, ES, GB, JP, KP, KR, NZ, SE				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2194478	AA	19960201	CA 1995-2194478	19950714
AU 9530985	A1	19960216	AU 1995-30985	19950714
AU 682125	B2	19970918		
EP 771321	A1	19970507	EP 1995-926694	19950714
R: DE, ES, FR, GB				
US 5712142	A	19980127	US 1996-604913	19960222

PRIORITY APPLN. INFO.:

US 1989-412434	19890926
US 1992-826089	19920127
US 1993-125115	19930921
EP 1990-914450	19900827
US 1994-276213	19940715
WO 1995-US8868	19950714

AB The gene encoding **Acidothermus** cellulolyticus E1 **endoglucanase** is cloned, sequenced, and expressed in heterologous microorganisms by std. recombinant DNA techniques. The 3004-bp sequence encodes a 562-amino acid precursor enzyme contg. a 41-residue signal sequence which is cleaved to yield the active E1 **endoglucanase** enzyme. New modified E1 **endoglucanase** enzymes can be produced by std. techniques of mutagenesis and mixed domain construction. The E1 **endoglucanase** is useful for hydrolyzing cellulose to sugars for simultaneous or later fermn. into alc.

L14 ANSWER 10 OF 10 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1987-08686 BIOTECHDS
TITLE: Bacterium produces thermostable cellulase; **Acidothermus** cellulolyticus enzyme characterization
AUTHOR: Seltzer R
LOCATION: (Pub. Address) American Chemical Society, 1155 Sixteenth Street NW, Washington D.C. 20036, USA.
SOURCE: Chem.Eng.News; (1987) 65, 18, 23-24

CODEN: CENEAR
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Scientists at Solar Energy Research Institute (SERI) in Golden, Colorado, have found a cellulase (EC-3.2.1.4) showing activity and stability at temperatures far higher than for any previously known cellulase. The enzyme is produced by the newly discovered **Acidothermus** cellulolyticus, and is potentially useful for economic fuel ethanol production from cellulosic biomass. The thermostable cellulase is also of interest in the food industry. A. cellulolyticus is moderately thermophilic and shows optimal cell growth at pH 5 and 55 deg. It is aerobic, utilizes a variety of substrates, and secretes a complex of cellulase enzymes. Crude culture broths from the bacterium show optimal temperatures of 75 deg for total cellulase activity and 83 deg for **endoglucanase** activity. At 95 deg, 38% of total activity and 60% of **endoglucanase** activity remain. Work is in progress regarding the isolation and characterization of the A. cellulolyticus cellulase enzymes, and the ultimate goal is the use of recombinant DNA technology to produce cellulase hyperproducing **mutants**. (0 ref)

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 2 DUP REM L3 (6 DUPLICATES REMOVED)

=> d l4 ibib ab 1-2

L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:323635 CAPLUS
DOCUMENT NUMBER: 137:43353
TITLE: Effect of single active-site cleft mutation on product specificity in a thermostable bacterial cellulase
AUTHOR(S): Rignall, Tauna R.; Baker, John O.; McCarter, Suzanne L.; Adney, William S.; Vinzant, Todd B.; Decker, Stephen R.; Himmel, Michael E.
CORPORATE SOURCE: Biotechnology for Fuels and Chemicals Division, National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO, 80401, USA
SOURCE: Applied Biochemistry and Biotechnology (2002), 98-100(Biotechnology for Fuels and Chemicals), 383-394
CODEN: ABIBDL; ISSN: 0273-2289
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutation of a single active-site cleft tyrosyl residue to a glycyl residue significantly changes the mixt. of products released from phosphoric acids-wollen cellulose (PSC) by EIcd, the catalytic domain of the **endoglucanase**-I from Acidothermus cellulolyticus. The percentage of glucose in the product stream is almost 40% greater for the **Y245G mutant** (and for an addnl. double **mutant**, **Y245G**/Q204A) than for the wild type enzyme. Comparisons of results for digestion PSC and of pretreated yellow poplar suggest that the obsd. shifts in product specificity are connected to the hydrolysis of a more easily digestible fraction of both substrates. A model is presented that relates the changes in product specificity to a mutation-driven shift in indexing of the polymeric substrate along the extended binding-site cleft.
REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:824394 CAPLUS
DOCUMENT NUMBER: 134:2062
TITLE: Acidothermus cellulolyticus E1 **endoglucanase variants Y245G**, Y82R and W42R with increased catalytic activity
INVENTOR(S): Himmel, Michael E.; Adney, William S.; Baker, John O.; Vinzant, Todd B.; Thomas, Steven R.; Sakon, Joshua; Decker, Stephen R.
PATENT ASSIGNEE(S): Midwest Research Institute, USA
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000070031	A1	20001123	WO 2000-US13971	20000519
WO 2000070031	C2	20020704		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,

MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1179051 A1 20020213 EP 2000-937647 20000519
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

US 2003054535 A1 20030320 US 2001-997504 20011119

PRIORITY APPLN. INFO.: US 1999-134925P P 19990519
WO 2000-US13971 W 20000519

AB The invention provides a method for making a glycosyl hydrolase characterized by an increase in catalytic activity on an insol. substrate. An active site assocd. glycosyl-stabilizing amino acid of the hydrolase is thus replaced with an amino acid, the replacing amino acid not strongly binding a disaccharide product in the active site. The method for making a glycosyl hydrolase characterized by an increase in catalytic activity on a sol. substrate comprises replacing a hydrophobic substrate binding amino acid of the hydrolase with a pos. charged amino acid. The invention specifically provides Acidothermus cellulolyticus E1 **endoglucanase variants**, comprising **Y42R**, **W82R**, or **Y245G**, and the DNA sequences encoding the enzymes. Ki values for inhibition of hydrolysis of 4-.beta.-D-cellobioside by native and **Y245G mutant** E1 indicate that the **mutant** catalytic domain binds cellobiose 15-fold less tightly than does the native enzyme, i.e., an increase in Ki from 2 to 30 mM cellobiose and a decrease in apparent binding energy of 1.7 kcal/mol.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT